National Institutes of Health
AIDS Vaccine Research Working Group (AVRWG)

Persistent Vector Workshop and Partnership for AIDS Vaccine Evaluation (PAVE)

January 12–13, 2005
Bethesda, Maryland

Meeting Summary

Introduction

Dr. Barton Haynes, Director of the Human Vaccine Institute, welcomed the meeting participants and announced three new working group members: Dr. Susan Buchbinder, Dr. James Wilson, and Dr. Joseph Sodroski (not present). Dr. Haynes stated that the purpose of the AIDS Vaccine Research Working Group (AVRWG) is to advise the National Institutes of Health (NIH), in particular, by providing an annual report on AIDS vaccine research. The discussions of this meeting would inform the 2005 report, scheduled to be released on April 15. This meeting would focus on the state of vector research, examining the current vector research pipeline, evaluating gaps in the research, and determining whether certain vectors should receive increased research emphasis.

Dr. James Bradac of the National Institute of Allergy and Infectious Diseases (NIAID) stated that the meeting also would consider vector persistence and funding for new-vector research. Dr. Bradac reviewed aspects of vaccine strategies and listed research areas currently funded. He encouraged the meeting participants to consider how the many vectors stack up with regard to immune-response safety, preexisting immune response, maximum insert size, level of antigen expression, and more. There is a need to define persistence and to consider evidence that persistence leads to strong, durable immunoresponse. Dr. Bradac encouraged the AVRWG members to consider prime/boost modalities, the use of head-to-head comparative studies, and whether the field is receiving ample support.

Immune Response to Persistent Antigen

Dr. Rafi Ahmed, Emory Vaccine Center

Dr. Rafi Ahmed described the kinetics of the humoral immune response in organ systems following an acute viral infection. He noted the effects of T-cell depletion and effects on plasmid cells. A vaccine must induce CD4 T-cells and sustain them. Mice studies have shown that systemic immunization is effective in producing effector T-cells in the spleen, which migrate to the gut. Only recently activated T-cells migrate effectively. Dr. Ahmed

stressed the need to determine the duration of antigen that is just right, producing fit T-cells but not persisting to the extent that important properties become exhausted. We must seek a balance between proliferative capacity and function. Live virus produces expansion and protection better than vaccination virus. Dr. Ahmed concluded that, for best results, one needs a massive hit to the immune system followed by low levels of antigen and periodic stimulation.

In discussion, the group agreed that DNA priming produces poorer results. They cautioned that there are differences (or at least inconsistencies in studies) between humans and nonhuman primates in the effects of boosting. We need better understanding of signaling and responses.

Adeno-Associated Virus Vectors
Dr. James Wilson, The Wistar Institute

Dr. Wilson reviewed the science of adeno-associated vectors (AAV), which can persist in muscle for years. AAV comprise a heterogeneous family that is dispersed widely in the human body. Sequence variations occur in the capsid proteins, and researchers have been studying the effects of capsid variation on vector performance. Recent research has involved using AAV to interject HIV-1 gag vectors into animal models and observing changes in CD8+ T-cells and B-cells. In discussion, Dr. Wilson stated that we need to learn more about the effects of preexisting immunity on the use of AAV. He suggested that AAV could be a good vector without being a good antibody.

Adeno-Associated Virus Vectors
Dr. Philip Johnson, Children's Hospital of Philadelphia

Dr. Johnson noted that AAV has never been associated with pathology. He described AAV's biology, dispersal in the body, and capacity for integration (which, in animal studies, his research group could not detect). In Dr. Johnson's studies, the vector DNA was found most often in tissue near the site of injection and in diffused tissue. Animal studies also showed that AAV (muscular injection with gag) antibodies persisted for many months, although ultimately at low plateau levels.

In discussion, Dr. Johnson noted that researchers have not looked for gag transcripts in lymph nodes. The effects of preexisting immunity on AAV are unknown, but it should be noted that only about 10 percent of persons have AAV antibodies. No human trials of AAV integration are planned at this point. Dr. Johnson suggested that the form of AAV he has been studying is episomal—it is created by recombinations, and the persisting cases are in non-dividing cells.

Herpesvirus Types 1 and 2 Vectors
Dr. David Knipe, Harvard Medical School

Dr. Knipe described research on the use of herpes simplex virus (HSV) as an AIDS vaccine vector. His group has been studying a replication-defective strain of herpes

simplex, which appears to stimulate neutralizing antibody responses in ways identical to those of replication-competent strains. Dr. Knipe described resulting reductions in viral growth over time following challenge. The virus has been shown to continually stimulate T-cells without reactivation, which remains unexplained. DNA/HSV was shown to produce the highest T-cell response. Dr. Knipe concluded that HSV vectors induce neutralizing antibodies, CD8+ T-cell response, and partial viral control; the magnitude of CD8+ response does not predict viral control; and TAT-specific CD8+ responses occur.

In discussion, Dr. Knipe noted that the research has not shown whether mucosal immunity is induced. So far, the researchers have seen persistence in mice models only (5 months). Over time, the virus products are found in the muscle area of application and not, for example, in innervating ganglion.

Gamma Herpesvirus Vectors
Dr. Ronald Desrosiers, Harvard Medical School

Dr. Desrosiers provided an overview of the family of herpesviruses, stating that researchers do not know which of them could be the best AIDS vaccine vector and all should be considered at this point. Advantages of these viruses include lifelong persistence of immune response and a large genome that accommodates foreign sequences. Dr. Desrosiers focused on gamma herpesvirus, which has a low prevalence, persists in B-cells, and displays a lack of pathogenicity in persons who are immunocompetent. Dr. Desrosiers and his colleagues inserted sequences into recombinant gamma herpesviruses, studied their effects in rhesus monkeys, and found antibody persistence at high levels.

In discussion, it was suggested that the researchers explore sex differences in the responses. There also is a need to perform persistence studies. Dr. Desrosiers said his group planned to study different sites and promoters and eventually human virus with deletions. The researchers have not addressed the effect of preexisting immunity.

Rabies Virus Vectors
Dr. Matthias Schnell, Thomas Jefferson University

Dr. Schnell characterized the rabies virus as not persisting, but long replicating and easy to modify. His group created a recombinant HIV strain of the virus and performed mice studies of immunogenicity. The group has performed safety studies, has worked to determine which strains of the virus reach the brain, and has compared cytolytic and noncytolytic viruses. The cytolytic version is stronger-acting in early days, whereas the noncytolytic version is stronger-acting in later periods. The researchers plan to consider longer periods and collect more long-term data. The vector response is potent, and boosting does not appear to be important. The researchers have not yet studied mucosal response.

Adenovirus Vectors
Dr. Marjorie Robert-Guroff, National Cancer Institute

Dr. Robert-Guroff characterized the adenovirus as not persistent but leading to good mucosal immune response. Studies in chimpanzees have found success in application of vectors followed by HIV challenge. Military vaccine programs have established safety of the adenovirus, and a Phase I human trial would begin soon. Another chimp study comparing replicating and nonreplicating strains found a much better response in the replicating group, including a better primary immune response and neutralizing ability.

In discussion, Dr. Robert-Guroff said that the upcoming human trials would be stratified, featuring sero-positive persons first and employing oral and intranasal routes of application. Group members encouraged Dr. Robert-Guroff to include less healthy individuals in the studies. Asked about the logistics of human studies, she replied that study participants who receive oral application would be allowed to go home, whereas participants who receive nasal applications would remain in the clinic until shedding stops. Asked whether a heterologous envelope virus would be used in the challenge study, Dr. Robert-Guroff responded that the virus to be used is partially heterologous.

Adenovirus Vectors
Dr. Hildegund Ertl, The Wistar Institute

Dr. Ertl also described advantages and disadvantages of using adenovirus vectors. She said that neutralizing adenovirus antibodies occur in 1 percent to 2 percent of the U.S. population. Her group is beginning to study zookeepers and other such workers to seek evidence of natural cross-species infection. Her work has suggested that low levels of expression might be an advantage, causing T-cells to act faster. She noted that integration studies are difficult. Her research group has not studied the relationship between mucosal and systemic immunization (a research gap to be filled).

SV40 Virus Vectors
Dr. David Strayer, Jefferson Medical College

Dr. Strayer listed characteristics of recombinant SV40 vectors, including the following: they are made at high titers; they transduce most of the cells responsible for generating the immune response; they integrate rapidly into resting or cycling cells; they express genes indefinitely; they provide low levels of protein production; they can be stored in a lyophilized form, they elicit no neutralizing antibodies; and they are safe. He stressed that researchers have yet to define a manner in which protective immunity against HIV would be measured. Dr. Strayer's research using mice has found that multiple injections boost responses and immunity is long-lived. He also has determined that SV40 can deliver a variety of HIV antigens with comparable results and can produce high levels of lysis.

In discussion, Dr. Strayer noted that his group has not performed a study without boosting. His group also has not studied the infectious pathway. He added that no researchers have determined how SV40 integrates.

Mycobacterium Vectors
Dr. William Jacobs, Howard Hughes Medical Institute

Dr. Jacobs described the mycobacterium BCG vaccine (bacillus Calmette-Guerin), which has been administered to newborn children for many years. The vaccine has demonstrated strong protection against tuberculosis in the United States and United Kingdom, but weaker protection elsewhere; for example, India. Dr. Jacobs described two BCG strains his research group made using TB with gene deletions. The group has been performing animal safety studies. The use of mouse models for HIV work has yet to occur, and Dr. Jacobs described various HIV vaccine strategies that he hopes to pursue. He also cited his work investigating a hypothesis that a protein called Nurim may prevent apoptosis; therefore, attempts to knock out this protein could lead to desired apoptosis. He noted that his group has seen immune differences between replicating and non-replicating forms of BCG.

Listeria Vectors
Dr. Elizabeth Hohmann, Massachusetts General Hospital

Dr. Hohmann described human studies using salmonella typhimurium and studies involving HIV-gag. She listed advantages of these vectors, such as their tendency to be taken up by most phagocytic cells (macrophages and dendritic cells) and great efficacy with a variety of viral antigens. However, these vectors can cause bacteremia and central nervous system infections. One study has suggested that preexisting immunity does not create a problem for the use of listeria vectors. Dr. Hohmann concluded that listeria viruses offer good research strategies and human studies are possible. In discussion, she noted that persistence (including antibodies) can extend beyond a year. Her studies have involved mucosal application, and she was unsure about a relationship to systemic effects.

Vesicular Stomatitis Virus
Dr. Stephen Udem, Wyeth Pharmaceuticals

Dr. Udem described efforts to create a sustained immunogenic response with Vesicular Stomatitis Virus (VSV), with measles virus serving as a model. VSV vector can be administered mucosally, can be manipulated readily, produces long-term antigen, and can target specific cell types. Nevertheless, questions remain. Can persistence be controlled? Can the vector be targeted effectively? Will vector immunity recur? The working group members noted an unanswered question about measles—does initial measles infection go on to prevent clinical measles or does it go on to prevent future infection?

Workshop Discussion and Recommendations

Dr. Haynes referred the group to a chart summarizing 6 key characteristics of each of the 12 vectors addressed in this meeting and indicating current gaps in knowledge. The characteristics were the following: providing a persistent immune response; affected by preexisting immunity; suitable as a prime vaccine; suitable as a boost; providing mucosal immunity from mucosal immunization; and providing mucosal immunity from systemic immunization. The group members reviewed the terms and responded "yes," "no," or "unknown" for each. Regarding the first characteristic, they noted that at least three types of possible persistence could be considered. This area is complicated. For example, it is possible to get a persistent immune state in the absence of antigenic stimulation. Overall, the chart revealed a large number of unknowns, indicating the need for research in many areas.

The cellular immune response involves a key question. Is it sufficient to create a pool of antibodies, or must there be persistent stimulation? Put another way, do we need persistent vectors or a persistent immune response (which can manifest in other ways).

Dr. Haynes also referred to a list of research gaps identified in the first part of this meeting (see the Appendix). Dr. Bradac provided an accounting of current support of research in the vector areas discussed at this meeting. Many of the vectors are supported by only one research grant.

Dr. Margaret Johnston, of NIAID, commented on a letter that the working group had sent to the institute, asking for an assessment of the AIDS portfolio. Dr. Johnston stated that the NIH Office of AIDS Research has asked all institutes to evaluate current AIDS research and current plans for future research. Further scrutiny of the portfolio is planned, as NIH seeks to determine whether ongoing research is appropriate. The institute is developing a strategic plan, incorporating ideas from President Bush's contributions at the upcoming G8 meeting, activities of the Gates Foundation, and more, including new resources.

Dr. Johnston stressed that funds would not be redirected from the vaccine portfolio. There is recognition of the need for such "little science." Dr. Sadoff cited the need to consider the use of "small manufacturing" for Phase I trials in the near future and the use of "distributed manufacturing" in larger, more distant efforts. Dr. Johnston responded that at least one award was recently made to a large manufacturer to support such a "distributed" strategy for supplying resources. Dr. Hunter agreed that the manufacturing potential is a key issue. Dr. Pensiero added that, nevertheless, most important are the finding and funding of good research ideas. Methods of manufacturing resources should be considered subsequently.

Dr. Corey called for a better filtering system that eliminates inherent biases and encourages good new research ideas. Dr. Nabel said that certain priorities are needed. Perhaps some research topics should be given strong funding, with smaller amounts of

money set aside for non-priority areas. Dr. Sadoff noted that some ideas—for example, usable cell lines—need to be considered early in planning research.

Dr. Haynes urged the group to suggest priorities without being dogmatic or overly exclusive. Dr. Watkins proposed emphasizing the funding of studies that vaccinate and challenge animals. Dr. Nabel stated the need to address critical path issues. The group members agreed that these issues are complex, for example, research areas are at different levels of development. How to winnow the choices? The group members agreed that it was too difficult to rank or prioritize at this meeting. The group could consider creating an algorithm that would be applied to research areas to help make go/no-go decisions.

Dr. Haynes said that a summary of this discussion would be incorporated into the working group's annual report. A summary of the discussion also would be incorporated into the annual report. Dr. Johnston proposed including ad hoc contributions to the ongoing discussion, including input by presenters at this meeting. Dr. Pensiero suggested holding another meeting to discuss other vectors, such as polio.

Dr. Haynes asked for volunteers to participate in a conference call to discuss critical path issues. Volunteers for the conference call included Drs. Haynes, Sadoff, Hammer, Nabel, Emilio, and Pensiero.

Program Update
Drs. James Bradac, Michael Pensiero, and Jorge Flores, NIAID

Dr. Bradac reviewed grants and contracts that have been awarded since the last meeting, including 16 Innovation grants, 3 partial HIVRAD awards, and 1 partial IPCAVD award. Dr. Sadoff wondered why AAV research was not receiving more funding. It is a promising, leading candidate for vaccine research. AAV research now is moving into the developmental stage—a stage, noted Dr. Sadoff, in which basic research is needed.

Dr. Haynes said again that the Workshop's report would note the need to prioritize vector research and would indicate problems with the peer review process. The group members further discussed the issue of ranking vaccine research areas and projects. This led Dr. Corey to propose the following resolution in the name of the working group:

Develop data on median and standard deviation priority scores of the solicited program project portfolio over several review cycles. Compare to median and standard deviation for the R01s. Fund program projects at score levels that meet, and preferably exceed, those of the R01s.

The working group members voted and unanimously accepted the resolution.

Dr. Pensiero described preclinical vaccine milestones within the division and characterized programs, such as the Vaccine Development Resources and Preclinical Master Contract, as working well. The programs are sufficiently mature that the division

can audit projects, and, in fact, some have been ended as a result. Dr. Pensiero listed vaccines now in the research pipeline (for example, Therion vaccines and a Wyeth vaccine) and studies that might be terminated (Thailand).

Dr. Flores provided an update on clinical trials, noting that 22 trials are active and 16 planned. Research issues being addressed include efficacy and laboratory readiness. A validation process is well under way. Dr. Flores listed a number of challenges to address, including appropriate assays, evaluation of immune-response breadth, evaluation of product combinations, prioritization of products, comparative studies, and safety assessments.

Summary of *Novel Assays for Assessing Nonhuman Primate T-Cell Responses*
Dr. Jeffrey Ahlers, NIAID

Dr. Ahlers reported on an October 2004 NIAID meeting that focused on new technologies—especially flow cytometry—that support immune studies, with a goal of identifying valid, discriminating assays. The meeting reviewed current methods for measuring immune responses and presented critical scientific questions; for example, what can operate as a correlate for immune protection?

Dr. Ahlers described the meeting's review of issues in polychromatic flow cytometry, including iterative processes, use of markers, and gating strategies. A conclusion of the meeting was that researchers employing these methods must perform tests to indicate what cells can be elicited. The meeting's participants agreed that researchers should study the GI tract or lung lavage to measure depletion of CD4 cells. They should observe responses in immunomucosal surfaces.

In discussion, Dr. Sadoff noted that, following vaccination, there is only a brief window to study transportation in the GI tract. Dr. Ahlers responded that this can depend on circulation patterns and certain technologies, such as in vivo imaging, might help.

Standard Virus Panels for Virus Neutralization Analysis
Dr. John Mascola, NIAID

Dr. Mascola reported on efforts by NIAID to establish standard virus panels for virus neutralization analysis. His group held a workshop on gauging neutralizing antibodies and published a commentary. A result of the discussions was development of a tiered strategy for selecting viruses, considering scientific questions, and modifying procedures as appropriate. Future efforts by Dr. Mascola's group would include publishing recommendations, creating panels for specific clades, establishing international networks, performing assay proficiency tests, and evaluating Phase III vaccine viruses against standard panels.

In discussion, Dr. Mascola said that one goal is to determine how broadly cross-reactive an antibody is. Dr. Sadoff suggested that a goal, therefore, should be to develop groupings of common epitopes. Dr. Nabel reminded the group that sensitivity to

neutralization must be correlated with immunity. Dr. Hunter encouraged the group to consider macrophage viruses. Dr. Buchbinder encouraged the group to study female responses.

Sample Availability from the VaxGen Trial
Dr. Jon Warren, NIAID

Dr. Warren reported on the effort to collect and store trial cells and plasma from the VaxGen study. His working group also is considering procedures for using the samples (receiving and processing requests). Tests have shown the collected samples to be viable. Dr. Warren noted scientific, serological, and cellular priorities being considered for the use of the samples. The working group and VaxGen would consider together their best use. Data subsequently collected from the use of the samples would be offered to NIAID for publication. Information on VaxGen samples would be found at the following site: www.niaid.nih.gov/reposit/over.htm

Update from the PAVE Lab Working Group
Dr. Richard Koup, NIAID

Dr. Koup reported on the PAVE Lab Working Group's efforts to study relevant laboratories in vaccine development and develop consistency in work such as blood processing, T-cell assay standardization, and peptide standardization. The group has developed a consensus document on processing, preservation, and shipping of samples and placed it on a Web site. It is performing a proficiency study, using frozen cell assays from different laboratories, and has found good results. For example, cytokine staining was found to be reproducible across labs. An October meeting addressed issues involving peptides, indicating a need to answer many questions, such as what minimum QA/QC requirements for peptide reagents are needed.

In discussion, Dr. Hunter and others suggested that multiple peptide pools would not be preferable. Standardized, comparable pools would lead to better results. The AVRWG could recommend their use, encouraging companies that might be reluctant. Dr. Sadoff agreed with this idea, but cautioned against overuse and overinterpretation of such pools. He also encouraged the Lab Working Group to consider simpler methods for collecting blood; for example, the use of finger sticks.

Update from the PAVE Site Development Working Group
Dr. Judith Wasserheit, Fred Hutchinson Cancer Research Center

Dr. Wasserheit stated goals of the Site Development Working Group, including the following:

- Determining elements of site capacity for efficacy trials
- Cataloging the status of existing capacity using the Web
- Determining recommendations for closing gaps

Strategies for closing gaps included training, administrative support, joint policy leadership, operational support by DAIDS, and follow up by agencies and organizations. Dr. Wasserheit reviewed a large number of site elements in terms of their need for each of those strategies. She listed future steps in the process—in particular, convening a meeting of CDC, DoD, HVTN, and IAVI representatives to plan for collaborative training.

Dr. Sadoff reminded the group that South Africa has a very strong review committee. DAIDS should not attempt to duplicate its operation.

Update from the PAVE Efficacy Trial Design Working Group
Dr. Steven Self, Statistical Center for HIV/AIDS Research and Prevention

Dr. Self reported on his working group's efforts to create procedures for establishing the qualification of vaccines and estimating take rates. The group has been considering methods such as region-specific and pooled analysis and the linking of trials. Dr. Self noted possible mathematical strategies for estimating take rates. He noted issues in trial designs—such as multicomponent vaccines and study populations spanning regions with different viral populations—and possible strategies for addressing them.

In discussion, Dr. Sadoff stated the need to power efficacy at 50 percent. Dr. Self agreed, for cases with low take rates. Dr. Wakefield wondered about cases in which products provide protection without correlating to the viral load. Dr. Self suggested designing larger trials for such cases.

The group members further discussed difficulties with trials conducted in different regions and systems. Dr. Johnston said that DAIDS has been struggling with this issue. Product sponsors must handle the regulatory issues, acting as a focal point. Dr. Nabel added that PAVE has developed some structures to increase harmonization. The working group members encouraged DAIDS to establish data-resource capabilities that lead to harmonization.

Dr. Sadoff and others encouraged the PAVE representatives to consider the PAVE study to be similar to a Phase III trial and to be prepared to find important results, such as validating a surrogate marker.

Adjournment

Dr. Hunter reminded the group that it would meet again on May 25–26, 2005. Topics for that meeting would include neutralizing antibodies, vector issues, a portfolio review (timelines and products), and the issue of making go/no-go decisions. Dr. Hunter adjourned the meeting.

Appendix

Research Gaps and Potential Actions

Persistent Vector Workshop

Main identified gaps in HIV-1 vector development:

- Need for vectors that induce robust primes in anti-HIV T- and B-cell responses
- Need for immunogens that boost primes for long-lasting B-cell induction
- Need for vectors that provide persisting antigen that is sufficient for maintaining robust CD4 and T-cell anti-HIV immunity
- Need for vectors and/or heterologous prime boosts that are not limited by preexisting immunity
- Need for vectors and/or heterologous prime boosts that induce both systemic and mucosal immunity

Other specific gaps/actions:

- Need to determine anti-AAV (and other vectors) antibody levels in developing communities
- Need to study and resolve regulatory issues involving AAV, gamma-2 herpesvirus, rabies virus, listeria, attenuated TB, aVsV, Single Cycle SIV, HSV, and EBV
- Need to determine all unknowns (and questioned entries) in the chart of vector characteristics

Additional areas to address:

- Define persistence
- Determine the duration of antigen that is “just right”—i.e., not too little, not too much (Goldilocks)
- Study possible sex differences (e.g., for gamma herpesvirus)
- Study possible reduced immunity persistence (gamma herpesvirus)
- For rabies virus, consider the nonlytic virus
- For adenovirus, study less healthy individuals
- For adenovirus, consider integration studies (although difficult)
- For cellular immune response, consider which of the following is sufficient: creating a pool of responders or providing persistent stimulation
- Consider the benefits/drawbacks of (1) small, individual manufacturing and (2) large, distributed manufacturing
- Consider other vectors (e.g., polio, macrophage viruses?)
- For the Standard Virus Panel effort, study groupings of common epitopes related to broad cross-reactions

- Develop data on median and standard deviation priority scores of the solicited program project portfolio over several review cycles; compare to median and standard deviation for the R01s; fund program projects at score levels that meet, and preferably exceed, those of the R01s.

Partnership for AIDS Vaccine Evaluation (PAVE)

- Consider developing standard peptide pools for research
- Consider simpler blood-drawing techniques (finger stick)
- Do not duplicate review processes that are already set up in African countries